# John Bacik

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# Enzyme detective

If Laboratory postdoc John-Paul (John) Bacik gets his wish, the world will significantly reduce its fossil fuel dependence in time for his young daughter's twenty-first birthday in 2035. Bacik is doing his part by contributing some of the molecular biology research necessary to make biofuels a cost#effective option.

"Plants and algae hold a lot of potential for abundant, renewable energy," Bacik says. "Although a variety of methods are being studied to use plants as a source of biofuel, I'm especially interested in looking at how we can do this with the help of enzymes—large protein molecules that enable and speed up biochemical reactions."

Amazing little power houses that they are, the enzymes Bacik works with convert components of plant walls or the inedible parts of plants into sugars that microorganisms can then transform into ethanol or other biofuels.

## The right tool for the job

To understand enzymes better, and perhaps later improve their efficiency through enzyme engineering, Bacik spends much of his time analyzing the molecules' three#dimensional structures.

"I'm part of the Protein Crystallography Station team at the Los Alamos Neutron Science Center," Bacik explains. "Crystallography is a form of high-resolution microscopy, and our station houses one of the world's premier neutron crystallography instruments."

In Bacik's case, the Protein Crystallography Station's neutron beam is directed at enzyme crystals and then scatters upon impact, producing a recordable pattern called diffraction.

"The diffraction is measured on a detector," Bacik notes, "and from this data I can determine the enzyme structure."

Interestingly, growing enzyme crystals is one of the more complicated, and certainly more time#consuming, aspects of Bacik's work.

## Crystal manufacture

Growing enzyme crystals can take Bacik anywhere from a few minutes to several months—or even longer—but begins with producing the initial enzyme itself with the help of the bacterium *Escherichia coli*, or *E. coli* for short. Despite other strains of *E. coli* getting a bad rap for causing disease, the laboratory strains Bacik uses are quite benign.

Bacik provides the *E. coli* with external genetic material and a “switch” that tells them to produce large volumes of Bacik’s enzymes of interest.

“I first take some frozen *E.coli* cells, let them thaw out a bit and use a micropipette to mix the cells with genetic material,” Bacik notes.

Bacik then puts the yellowish liquid in a bucket of ice for 20 minutes, followed by a bath in hot water.

“The temperature change, known as heat-shock, momentarily changes the outer layers of the *E. coli* and makes them more permeable to the introduced DNA,” Bacik says. “The DNA in turn provides the instructions to produce the enzyme I want."

Once Bacik has enough material, he extracts the enzyme using ultrasonic frequencies to break the *E. coli* cells open, and then he purifies the enzyme even further with additional machines. He finally concentrates the enzyme in a centrifuge and mixes the resulting sample with a liquid that promotes crystallization.

Like all protein crystals, enzyme crystals generally only grow large enough to be viewed under a microscope. Bacik, and other researchers using neutron crystallography, have the extra challenge of growing crystals to a size sufficient enough to scatter neutrons, which translates to a size visible to the naked eye.

*A tiny, white crystal can be seen near the top of the capillary tube, while a yellow plug of wax seals the end.*

“As a result of enzymes’ inherent flexibility and strange shapes, they are not all that amenable to this additional growth,” Bacik suggests, “so I test many different conditions and watch what effects they have on the crystal formation.”

Bacik also tries to improve the enzymes’ appearance by adjusting some of the surrounding conditions, such as the pH value and salt concentration.

“Visual appearance is often an indicator of the underlying order of the crystal, but not always,” Bacik says. “Sometimes it’s the ugly crystals that work out better.”

## Birthday planning

One of the ways to preserve protein crystals until they are ready to encounter the Protein Crystallography Station's neutron beam is to freeze them in liquid nitrogen.

“Back in Winnipeg, Canada, where I’m from, one of the ongoing jokes was that we could just stick the crystals outside, because it was so cold,” Bacik laughs. “In a typical Winnipeg winter it’s not uncommon for the temperatures to plummet to minus 30 degrees or below.”

Bacik has been interested in science from a very young age. While many of his friends enjoyed watching cartoons on TV, Bacik couldn’t get enough of the science show NOVA.

“When my father, a teacher, asked me one evening what I wanted to be when I grew up, I blurted out ‘Charles Darwin’ without thinking,” Bacik recalls with a smile.

Bacik’s 18-month-old daughter, Christiane, has been part of Bacik’s work life from the day she was born. Two weeks before Christiane was expected, Bacik was in his office preparing a biofuels-related grant proposal when his wife went into labor. Today, the background image on Bacik’s cell phone is a photo of his wife and baby Christiane, with Christiane enthusiastically clapping her hands.

“By the time Christiane turns 21 and pulls up to a gas station,” Bacik says, “I hope that she will be able to fill her car with a biofuel that I helped develop.”

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Bacik works for the Bioscience Division’s Bioenergy and Biome Science group.

*The banner photo at the very top shows the Protein Crystallography Station's semicircular, shiny neutron diffraction detector to Bacik's right.*

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